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TITLE: Vascular and Skeletal Muscle Function in Gulf War Veterans Illness

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CONTRACTING ORGANIZATION: Boston VA Research Institute, Inc.
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14. ABSTRACT Gulf War Illness (GWI) is a constellation of symptoms including fatigue, musculoskeletal pain, memory loss, and mood changes reported by Gulf War Veterans shortly after their return in 1991. Approximately 40% of Gulf War Veterans (over 1/4 million Veterans) have GWI by the Center for Disease Control criteria for GWI (a recommended method for defining GWI). The underlying causes of GWI are poorly understood. The overall goal of our study is to determine if there are differences in blood vessels, skeletal muscle performance, and their controlling proteins and genes in Gulf War Veterans with and without GWI. Abnormalities in these factors may explain the symptoms of fatigue and muscle pain that are major parts of GWI. These insights could lead to new treatments for GWI as well as other illnesses with similar symptoms. Our pilot data show that we can assess blood flow to muscle, muscle strength and fatigue and examine proteins and genes from a specimen of muscle in Gulf War Veterans. We will assess if abnormalities in these factors are potential explanations for GWI. This study is looking to enroll 70 Veterans (35 with GWI and 35 without GWI) and is currently open to enrollment.					
15. SUBJECT TERMS Gulf War Syndrome, Persian Gulf Syndrome/physiopathology, Veterans					
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1. INTRODUCTION: Gulf War Illness (GWI) is a constellation of symptoms including fatigue, musculoskeletal pain, and neurocognitive reported by Gulf War Veterans shortly after their return from deployment in 1991. The Center for Disease Control and Prevention (CDC)'s clinical diagnostic criteria for GWI is one of two recommended by an Expert Committee, and is based on symptoms in three categories: fatigue, mood/cognition, and musculoskeletal symptoms. Currently, approximately 40% of Gulf War Veterans (over ¼ million Veterans) have GWI by these criteria. The pathophysiological mechanisms underlying GWI are not understood and insights into these mechanisms could lead to new treatment interventions. Furthermore, abnormalities in peripheral blood flow related to endothelial function and muscle bioenergetics due to environmental toxins, such as those present in the Gulf War, are plausible mechanisms that could relate to the musculoskeletal symptoms of GWI. This study will determine the pathophysiology, and related genome and transcriptional mechanisms related to endothelial function and muscle mitochondrial biogenesis in Veterans with and without GWI through a case-control design of 70 Veterans who have served in the Gulf War and are participants of the ongoing Fort Devens Cohort. Specific aims include comparisons of: (1) microvascular endothelium-dependent and endothelium-independent function of the profunda femoral artery using techniques commonly used for peripheral endovascular interventions, (2) peak oxygen uptake and ventilator anaerobic threshold during cardiopulmonary exercise testing and other muscle functions, (3) expression of genes relevant to endothelial function and mitochondrial function in muscle biopsy samples, and (4) gene polymorphisms related to endothelial and mitochondrial respiratory function.

2. KEYWORDS:

Gulf War Syndrome

Persian Gulf Syndrome/physiopathology

Veterans

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Major Tasks	Timeline (months)	Status
Major Task 1: Institutional Review Board (IRB) Approval		
Modify the current protocol to add new experiments and aim (microarray assays and next-generation RNA sequencing)	0.5	Completed.
Submit final protocol to VA Boston Healthcare System (VABHS) IRB	0.5	Completed.
Milestone: Achieve local IRB approval of protocol	1	Received VA Boston IRB approval for modified protocol amendment, Protocol Version 2.0 on 11-JAN-2016.
Major Task 2: Recruitment of Subjects		
Send batch invitations to 400 Gulf War Veterans who have completed the Fort Devens cohort study	1-24	Submitted to and received HRPO Initial Approval for protocol (version 2/ dated 23-

		Dec-2015) on 09-FEB-2016. Dr. Maxine Krengel (Co-Investigator) helped initiate recruitment by sending Gulf War Veterans invitation letters to participate around 19-FEB-2016. As of 30-JUN-2016, 75 Veterans have received a letter of invitation to consider study participation.
Major Task 3: Endothelial Function Study and Muscle Biopsy		
Schedule Visit 1 (Endothelial function studies and muscle biopsies)	1-24	As of 30-JUN-2016, 5 Veterans have replied with interest in study participation and have been scheduled for Visit 1.
Complete endothelial function studies and muscle biopsies and measurement of intravascular ultrasound and flow data to assess microvascular and conduit endothelial function	1-28	Pending
Milestone: Complete endothelial function data and muscle biopsies on 70 subjects	28	Pending
Major Task 4: Exercise and Cardiopulmonary Stress Testing		
Schedule Visit 2 (Exercise and cardiopulmonary stress tests)	3-28	As of 30-JUN-2016, since Visit 2 must occur at least 2 weeks after completion of Visit 1, scheduling of Visit 2 is pending completion of Visit 1 for all 5 Veterans who have expressed interest in study participation.
Complete exercise and cardiopulmonary stress studies and interpretation	3-28	Pending
Milestone: Complete exercise data on 70 subjects	28	Pending
Major Task 5: Histopathology and Electron Microscopy		
Prepare muscle biopsy specimens for histopathology and electron microscopy and image	4-30	Pending
Complete data on muscle analysis including histopathology	4-30	Pending
Milestone: Complete histopathological data and electron microscopy data on representative subjects	30	Pending
Major Task 6: Gene and protein expression relating to mitochondrial biogenesis		
Isolate DNA, RNA, and protein from muscle tissue samples. Prepare cDNA from RNA samples.	4-28	Pending
Complete qPCR and Western Blot to assess genes and proteins regulating mitochondrial biogenesis.	4-30	Pending

Milestone: Complete data on specific genes and proteins regulating mitochondrial biogenesis on 70 subjects	30	Pending
Major Task 7: Transcriptome microarrays comparing cases and controls		
Run microarrays at Dana Farber Microarray Core Lab from cDNA samples	24-30	Pending
Interpret results and identify candidate genes related to Gulf War Illness	24-30	Pending
Milestone: Complete analysis of transcriptome microarray data on 70 subjects	30	Pending
Major Task 8: SNP Microarray		
Run MVP microarray at Dana Farber Microarray Core Lab	4-28	Pending
Identify candidate genetic polymorphisms related to GWI	4-30	Pending
Milestone: Complete data analysis of SNP microarray data on 70 subjects	30	Pending
Major Task 9: Finalize data analysis, present results and meetings, publish results		
Complete statistical analyses including comparisons of cases and controls and prepare for publication, presentation, and public release of de-identified data for other researchers.	24-36	Pending

What was accomplished under these goals? This report summarizes the research progress in the most recently completed budget period from July 1, 2015 to June 30, 2016. This time period corresponds to the first of this three-year project. The objectives of this study is to investigate the hypothesis that when compared to Veterans without Gulf War Illness (GWI), Veterans with GWI will have differences in arterial endothelial function, muscle function determined by cardiopulmonary exercise testing, and expression of genes responsible for mitochondrial function. This is a case control study of 2 visits looking to enroll 70 participants (35 with GWI and 35 without GWI) from a well characterized cohort of Gulf War Veterans (the Fort Devens study). Study Visit 1 consists of an endothelial function test performed using standard cardiac catheterization techniques used for peripheral artery interventions, and a muscle biopsy of the vastus lateralis muscle. Study Visit 2 consists of cardiopulmonary exercise testing and other tests of muscle strength and endurance.

The first half of the budget period was focused on amending the study protocol to include new experiments (microarray assays and next-generation RNA sequencing) to meet Specific Aims 3 and 4 and obtaining approval from the VA Boston Healthcare System Institutional Review Board (IRB) (local) and the U.S. Army Medical Research and Material Command (USAMRMC) Human Research Protection Office (HRPO). The study protocol (version 2/dated 23-Dec-2015) was approved by the VA Boston Healthcare System IRB on 11-Jan-2016. The USAMRC, Office of Research Protections (ORP), HRPO reviewed the protocol and found that it complies with applicable DOD, US Army, and USAMRMC human subject protection requirements and recommended initial approval on 09-Feb-2016.

Subject recruitment commenced shortly afterwards with the first batch of letters of invitations sent to Gulf War Veterans of the Fort Devens cohort on 19-Feb-2016. As of 30-JUN-2016, 75 Veterans have received a letter of invitation to consider study participation with 5 responding with interest. Visit 1 has been scheduled for all five Veterans, with appointments in July through September 2016. Batches of invitation letters continue to be mailed every 2-3 weeks. Additionally, efforts to inform the Gulf War Veteran community of the study were completed through distribution of study brochures and tabling at the 25th Gulf War Anniversary Reunion hosted by the VA Boston Healthcare System and Boston University School of Medicine on 09-Apr-2016. These quick initial responses give a good indication of the large interest in scientific research participation in this community.

What opportunities for training and professional development has the project provided?
Nothing to Report.

How were the results disseminated to communities of interest? Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals? In the next reporting period, we are planning to continue subject recruitment through invitation letters to Veterans of the Fort Devens cohort. The Fort Devens cohort consists of over 1300 Veterans. Veterans who do not respond to the initial letter will be mailed up to 2 reminder letters. Study Visit 1 and 2 will be scheduled and completed for all Veterans expressing interest in study participation.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?
Fatigue and musculoskeletal symptoms are major components of GWI and could have an important impact on other symptoms associated with GWI. There are plausible reasons why endothelial function and mitochondrial biogenesis in muscle may be affected by exposure to environmental toxins during the Gulf War and lead to these symptoms.

In particular, pyridostigmine and nerve gases are anticholinesterase agents that potentially have long term effects on the balance of cholinesterases and acetylcholine, which could affect activity at the neuromuscular junction of skeletal muscle, muscarinic receptors affecting vascular smooth muscle tone, and damage mitochondrial structure and electron transport activity in several tissues including muscle.

Insights on the pathogenesis of GWI could lead to new treatments for GWI, but also provide novel mechanistic insights into other exposure-related occupational health illness, such as pesticide exposure in the agricultural industry. Our study may also elucidate mechanisms of interest that require investigation as causes of other illnesses with muscle fatigue, pain, and abnormal muscle metabolism, such as peripheral artery disease and chronic heart failure, and advance our understanding of the pathophysiology of GWI and discover molecular pathways that could elucidate novel treatments for GWI. It may also direct future research into abnormalities of important molecules that could form the basis of an improved diagnostic test, although establishing a diagnostic test is not the focus of this proposal.

As the study is in the recruitment phase, there are currently no findings to report.

What was the impact on other disciplines? Nothing to Report.

What was the impact on technology transfer? Nothing to Report.

What was the impact on society beyond science and technology? Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change: Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them: Delays in IRB and HRPO approval may have affected progress in subject recruitment. We will continue to monitor rate of subject recruitment closely and propose changes in the recruitment plan when necessary.

Changes that had a significant impact on expenditures: Sara Temiyasathit Jones PhD left the VA Boston Healthcare System and is no longer able to serve as Co-Investigator for this project. Request to remove Dr. Jones as key personnel was submitted with the last quarterly technical report due on 16-Apr-2016. Her original project management responsibilities will be replaced through hire of a senior research individual with experience in overseeing and managing clinical research projects. This individual will be starting on 01-Aug-2016.

Additionally, Dr. Jones's expertise in genetics and cellular and molecular biology is replaced through collaboration with Calum MacRae MD PhD at Brigham and Women's Hospital. Dr. MacRae has extensive experience in molecular biology techniques, including RNA and DNA isolation and analysis, protein isolation expression, microchip arrays, and next generation RNA sequencing. He will serve as a collaborator and no salary will be taken from this project.

These changes do not adversely affect the progress of this study but is expected to change expenditures. Salary support was requested for Dr. Jones in the original budget. We anticipate this allocation will be used to fund the replacement senior research individual beginning 01-Aug-2016.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to Report.

6. PRODUCTS: Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project? Individuals who have worked on this project during the most recent budget period are described below with their efforts and contribution divided by each quarterly reporting period.

July 1, 2015 to September 30, 2015

<i>Name:</i>	Scott Kinlay, MBBS, PhD
<i>Project Role:</i>	Principal Investigator
<i>Research Identifier:</i>	0000-0001-7687-9136
<i>Nearest person month worked:</i>	1
<i>Contribution to Project:</i>	Dr. Kinlay has made revisions to the most current protocol to include new experiments (microarray assays and next-generation RNA sequencing), change in exclusion criteria to increase enrollment rates, and expanded allowable time between Visit 1 and 2 to allow for scheduling. He has also identified a Research Assistant to work on this study.

<i>Name:</i>	Sara Temiyasathit Jones, PhD
<i>Project Role:</i>	Co-Investigator
<i>Research Identifier:</i>	0000-0002-8882-7744
<i>Nearest person month worked:</i>	1
<i>Contribution to Project:</i>	Dr. Jones is overseeing the conduct of this project, contributing specifically to developing plans for collection and storage of muscle biopsy samples.

<i>Name:</i>	Margot Quinn, BA
<i>Project Role:</i>	Research Assistant
<i>Research Identifier:</i>	N/A
<i>Nearest person month worked:</i>	2
<i>Contribution to Project:</i>	Ms. Quinn has organized data from vascular studies, exercise studies and molecular studies collected on subjects (n=19) enrolled in the pilot study. She has also coordinated meetings between Senior Study personnel.

October 1, 2015 to December 31, 2015

<i>Name:</i>	Scott Kinlay, MBBS, PhD
<i>Project Role:</i>	Principal Investigator
<i>Research Identifier:</i>	0000-0001-7687-9136
<i>Nearest person month worked:</i>	1
<i>Contribution to Project:</i>	Dr. Kinlay revised the most current protocol (version 2.0, dated 23-DEC-2015) to address clarifications requested by HRPO. This also includes identifying a DoD independent Research monitor and clarifying plans to complete muscle and tissue analysis.

<i>Name:</i>	Sara Temiyasathit Jones, PhD
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<i>Project Role:</i>	Co-Investigator
<i>Research Identifier:</i>	0000-0002-8882-7744
<i>Nearest person month worked:</i>	1
<i>Contribution to Project:</i>	Dr. Jones is overseeing the conduct of this project and continues to develop plans for collection and storage of muscle biopsy samples.
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<i>Name:</i>	Margot Quinn, BA
<i>Project Role:</i>	Research Assistant
<i>Research Identifier:</i>	N/A
<i>Nearest person month worked:</i>	1
<i>Contribution to Project:</i>	Ms. Quinn has been working on developing a handbook that details all study conduct and operations. She continues to coordinate meetings between senior Study Personnel.
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<i>Name:</i>	Samantha Ly, MA
<i>Project Role:</i>	Program Manager
<i>Research Identifier:</i>	0000-0003-1994-7627
<i>Nearest person month worked:</i>	3
<i>Contribution to Project:</i>	Ms. Ly assisted with protocol revisions and changes requested by HRPO and liaised between Senior Study Personnel and HRPO staff. She also reviewed previously collected data and worked on data cleaning.
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<i>Name:</i>	Jacquelyn-My Do, MPH
<i>Project Role:</i>	Assistant Program Manager
<i>Research Identifier:</i>	N/A
<i>Nearest person month worked:</i>	1
<i>Contribution to Project:</i>	Ms. Do was responsible for administrative tasks including maintenance of study regulatory binders, tracking study equipment inventory, and revising case report forms.
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January 1, 2016 – March 31, 2016	
<i>Name:</i>	Scott Kinlay, MBBS, PhD
<i>Project Role:</i>	Principal Investigator
<i>Research Identifier:</i>	0000-0001-7687-9136
<i>Nearest person month worked:</i>	3
<i>Contribution to Project:</i>	Dr. Kinlay is overseeing subject recruitment since IRB and HRPO approval and is looking to hire a more senior research individual to supervise the Research Assistant as recruitment begins and to assume the administrative project management responsibilities (e.g. project oversight) of Dr. Sara Jones since her departure from this project.
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Name:	Maxine Krengel, PhD
Project Role:	Co-Investigator
Research Identifier:	N/A
Nearest person month worked	1
Contribution to project:	Dr. Krengel has begun subject recruitment by mailing out letters of invitation to Gulf War Veterans in the Fort Devens cohort.

April 1, 2016 – June 30, 2016 (most recent)

Name:	Scott Kinlay, MBBS, PhD
Project Role:	Principal Investigator
Research Identifier	0000-0001-7687-9136
Nearest person month worked:	1

Contribution to Project:	Dr. Kinlay is overseeing subject eligibility prior scheduling appointments to ensure they meet the eligibility criteria. Supervise the Research Assistant as recruitment begins and to assume the administrative project management responsibilities (e.g. project oversight) of Dr. Sara Jones since her departure from this project.
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Name:	Margot Quinn, BA
Project Role:	Research Assistant
Research Identifier:	N/A
Nearest person month worked:	1
Contribution to Project:	Ms. Quinn assisted Dr. Kinlay and Dr. Krengel with subject recruitment, starting with attendance at the 25 th Gulf War Anniversary reunion. She has also prepared letters of invitation to 75 Veterans and triaged calls from interested participants.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Nothing to Report.

What other organizations were involved as partners? In the next reporting periods, depending on the rate of subject recruitment, we may decide to begin analysis of muscle tissues. This will include isolation and analysis of RNA and DNA, protein isolation expression, microchip arrays, and next generation RNA generation. We plan to work with our collaborator, Dr. Calum MacRae at the Brigham and Women's hospital to complete these analyses.

Organization Name: Brigham and Women's Hospital

Location of Organization: Boston, MA

Partner's contribution to the project: Collaboration

Until sample analysis commences, we current do not have any partner organizations.

8. **SPECIAL REPORTING REQUIREMENTS COLLABORATIVE AWARDS:** None.

9. **APPENDICES:** Please see the attached quad chart.

Vascular and Skeletal Muscle Function in Gulf War Veterans Illness

Log Number: GW14003

Award Number: W81XWH-15-1-0216

PI: Scott Kinlay, MBBS, PhD

Org: Boston VA Research Institute, Inc. (BVARI)

Award Amount: \$870,642.00

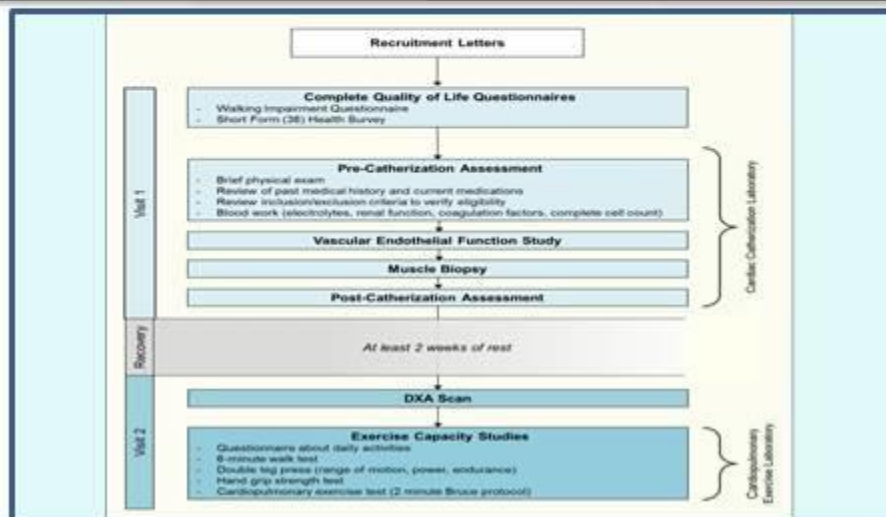


Study/Product Aim(s)

- To determine if microvascular endothelium-dependent and endothelium-independent function of the profunda femoral artery is impaired in subjects with Gulf War Veterans Illness (GWVI) compared to deployed Veterans without GWVI.
- To determine if peak oxygen uptake and ventilatory anaerobic threshold during cardiopulmonary exercise testing, and other muscle functions are impaired in subjects with GWVI compared to deployed Veterans without GWVI.
- To determine how the expression of genes relevant to endothelial function and mitochondrial function in muscle biopsy samples differs between subjects with GWVI compared to deployed Veterans without GWVI.
- To determine if polymorphisms to genes relating to endothelial function and mitochondrial respiratory function differ between subjects with GWVI compared to deployed Veterans without GWVI.

Approach

Gulf War Illness (GWI) is a constellation of symptoms including fatigue, musculoskeletal pain, and neurocognitive dysfunction reported by Gulf War Veterans shortly after their return from deployment in 1991. There are plausible reasons why endothelial function and mitochondrial biogenesis in muscle may be affected by exposure to environmental toxins during the Gulf War and lead to GWI symptoms. We hypothesize that compared to Veterans without GWI, Veterans with GWI will have differences in arterial endothelial function, muscle function determined by cardiopulmonary exercise testing, and the expression of genes responsible of mitochondrial function.



Accomplishment: This IRB-approved prospective cross-sectional clinical trial will consist of 2 study visits. 70 Gulf War Veterans (35 with GWI and 35 without GWI) will be enrolled.

Timeline and Cost

Activities	CY	15	16	17	18	19
Milestone 1: Achieve local IRB approval of protocol						
Milestone 2: Complete Visit 1 (endothelial function and muscle biopsies) on 70 subjects						
Milestone 3: Complete Visit 2 (exercise and cardiopulmonary stress test) on 70 subjects						
Milestone 4: Complete histopathology and electron microscopy analysis						
Milestone 5: Complete gene and protein analysis						
Milestone 6: Complete analysis on transcriptome microarray data						
Milestone 7: Complete analysis of SNP microarray data						
Finalize data analysis, present results and meetings, publish results						
Estimated Budget (\$K)		\$0	\$220	\$361	\$287	\$0

Updated: 30-JUN-2016

Goals/Milestones

CY15 Goals – Institutional Review Board (IRB)

- ☒ Achieve local IRB approval
- ☒ Achieve HRPO approval

CY16/17 Goals – Subject Recruitment

- ☒ Start recruitment with letters of invitations
- ☐ Schedule and conduct Visits 1 and 2

CY18 Goals – Complete recruitment and data analysis

- ☐ Complete Visits 1 and 2 on 70 Subjects
- ☐ Complete histopathological data, electronic microscopy data, specific genes and proteins regulating mitochondrial biogenesis, analysis of transcriptome microarray data on samples collected

CY19 Goal – Analyze and publish results

- ☐ Analyze, present, and publish results at DoD and scientific meetings

Comments/Challenges/Issues/Concerns: N/A

Budget Expenditure to Date

Projected Expenditure: \$220,000; Actual Expenditure: \$1,000